REMARKS

1. Preliminary Matters

a. Status of the Claims

Claims 69-72 and 89-96 are pending and under active consideration. Claims 69 and 89 are amended. Applicant requests entry of the amendments and remarks made herein into the file history of this application. Upon entry of the amendments, claims 69-72 and 89-96 will be pending and under active consideration.

b. Amendments to the Claims

Claims 69 and 89 are amended to recite "RNA encoded by" instead of "RNA equivalent." Support for the encoded RNAs can be found at paragraphs 0031 and 0179 of the specification as originally filed.

2. Remarks

a. 35 U.S.C. § 112, 1st paragraph

On pages 2-5 of the Office Action, the Examiner rejects claims 69-72 under 35 U.S.C. § 112, first paragraph, as allegedly containing new matter. Specifically, the Examiner asserts that the application does not provide support for "an RNA equivalent of (a)" as indicated in claim 69. Amended claims 69 and 89 do not recite "RNA equivalent," but instead recite "RNA encoded by (a)." The Examiner acknowledges at page 4 of the Office Action that Applicant has express possession of RNA sequences encoded by the claimed DNA sequences. In view of the foregoing, Applicant respectfully requests that the Examiner reconsider and withdraw the rejection of claim 69 under 35 U.S.C. § 112, first paragraph.

b. 35 U.S.C. §§ 101 and 112

On pages 5-10, the Examiner rejects claims 69-72 under 35 U.S.C. § 101 because the claimed invention is allegedly not supported by either a credible, specific and substantial asserted utility, or alternatively, a well established utility.

In order to satisfy the utility requirement, a specific and substantial utility must either (i) be cited in the specification or (ii) be recognized as well as established in the art, and the utility must be credible. *See In re Fisher*, 421 F.3d 1365, 1371 (Fed. Cir. 2005) and *Revised Interim Utility Guideline Training Materials* ("Guidelines").

(1) Specific Utility

A specific utility is a utility that is specific to the particular claimed subject matter, which is in contrast to a general utility that would be applicable to a broad class of inventions. *See Fisher*, 421 F.3d at 1371 and *Guidelines*. Applicant respectfully submits that the application provides a specific utility for the claimed miRNA-related nucleic acids in accordance with *Fisher* and *Guidelines*.

In *Fisher*, the claims at issue were directed to five (5) out of more than 32,000 EST that were disclosed in the application. Each of disclosed ESTs were from a cDNA library of pooled leaf tissue isolated from a maize plant. The *Fisher* application did not disclose the location of the ESTs in the genome or the function of the underlying genes. *Fisher* asserted that the utilities for claimed ESTs were (1) serving as a molecular marker; (2) measuring the level of mRNA in a tissue sample; (3) providing a source of primers for PCR of specific genes; (4) identifying the presence or absence of a polymorphism; (5) isolating promoters via chromosome walking; (6) controlling protein expression; and (7) locating genetic molecules of other plants and organisms. *See Fisher*, 421 F.3d at 1367-1368. It is important to note that each of the utilities asserted were not limited to any specific gene, genetic location or protein.

The *Fisher* court concluded that the asserted utilities were clearly not "specific." The court explained that any EST transcribed from any gene in maize could perform the seven uses such as being a molecular marker, a primer, or measure the level of RNA in a tissue sample. In other words, nothing about the seven alleged uses separated the claimed ESTs from the vast number of other ESTs also disclosed in the application. The keystone to the lack of specific utility in *Fisher* is that the claimed ESTs **did not correlate to an underlying gene of known function found in the maize genome**.

Similar to *Fisher*, the current application discloses a large number of nucleic acid sequences. In stark contrast to *Fisher*, however, the instant application provides that each of the disclosed nucleic acids may be used to target and modulate expression of **specific** gene transcripts. Table 7, lines 188050-188054 and Table 8, lines 660704-660717 of the application disclose that the claimed miRNA-related sequences specifically target mRNA transcripts of the target gene ATP7A. Additionally, Table 7, lines 253472-253431 and Table 8, lines 843989-844008 disclose that the claimed miRNA-related sequences also specifically target mRNA transcripts of the target gene STAT3. Consequently, the claimed nucleic acids are of a **specific and unique nature** because these nucleic acids regulate the translation of mRNAs from the **specific target genes ATP7A and STAT3**. Accordingly, the asserted utility of the claimed invention is not vague or meaningless, and there is a well-defined public benefit to regulating ATP7A and STAT3.

(2) Substantial Utility

To satisfy the "substantial" utility requirement, it must be shown that the asserted use of the claimed invention has a significant and presently available benefit to the public. *See Id.* at 1371 and *Guidelines*. Applicant respectfully submits that the application provides a substantial utility for the claimed miRNA-related nucleic acids in accordance with *Fisher* and *Guidelines*.

In *Fisher*, it was admitted that the underlying genes for the ESTs had no known function. *Fisher* argued that this was irrelevant because the seven asserted uses (discussed above) were not related to the function of the underlying genes. Importantly, *Fisher* failed to provide any evidence that any of the claimed ESTs could be used for any of the asserted uses. Consequently, the *Fisher* court concluded that

the claimed ESTs were "mere 'objects of use-testing,' to wit, objects upon which scientific research could be performed with no assurance that anything useful will be discovered in the end." *See Fisher*, 421 F.3d at 1373, *quoting Brenner v. Manson*, 383 U.S. 519 (1966).

In further sharp contrast to *Fisher*, the present application discloses that the claimed nucleic acids may be used to bind and regulate mRNA transcripts of ATP7A. *Instant Application,* Table 8, lines 660704-660717. In addition, ATP7A[1] is known to be a copper-transporting, P-type ATPase that is present in most non-hepatic tissues in humans. *See* Petris M.J. *et al.* Jo Biol Chem. 2002;277(48):46736-42. Mutations in the ATP7A gene cause defects in copper export from intestinal cells and aberrant distribution of copper around the body, which results in a systemic copper deficiency with associated symptoms, including severe neurological and connective tissue defects, impaired temperature control, and hypopigmentation. *Id.* The ATP7A protein transports copper to the lumen of secretory compartments to provide copper to various copper-dependent enzymes, and in the presence of copper, traffics to the plasma membrane where it maintains copper homeostasis via copper efflux. *Id.* Accordingly, ATP7A expression could be modulated to alter copper export.

STAT3 is known to be a latent transcription factor that becomes activated by phosphorylation in response to extracellular ligands. *See* Bromberg J. F. *et al.* Cell 1999;98:295-303. STAT3 proteins form active dimers that accumulate in the nucleus and activate specific gene transcription. *Id.* STAT3 is constitutively activated in *src*-transformed cells, as well as in multiple myeloma cells that have become growth factor independent. *Id.* Overexpression of a constitutively active STAT3 protein with only two amino acid substitutions in mammalian cells is sufficient to transform the cells. *Id.* These cells are tumorigenic when injected into nude mice. *Id.* Accordingly, STAT3 expression could be modulated to alter cell cycle regulation.

The evidence described above clearly supports that the claimed nucleic acids have a number of presently available benefits to the public. Such benefits are the ability to modulate the expression of ATP7A or STAT3 in order to alter copper export or cell cycle regulation, respectively. In view of the application providing particular targets of known function for the claimed miRNA-related nucleic acids, Applicant respectfully submits that the specific and substantial utility requirements are satisfied in accordance of *Fisher* and *Guidelines*.

(3) Credible Utility

On page 7 of the Office Action, the Examiner alleges that there is no evidence to suggest that the claimed nucleic acids are expressed in any cell whatsoever. Applicant respectfully submits that the reference Zanetta D.L. *et al.* Brazilian Journal of Medical and Biological Research 2007;40:1435-40

[1] ATP7A is also referred to in the art as Menkes protein, or MNK.

("Zanetta") establishes that the miRNAs with the sequences set forth in SEQ ID NO: 2 (hsa-miR-20) and SEQ ID NO: 9 (hsa-miR-18) are expressed. These miRNAs are produced by the hairpins with SEQ ID NOs: 140732 and 140670, respectively, which are produced by SEQ ID NO: 142700. Specifically, Zanetta analyzes expression of miRNAs that are upregulated in cells derived from patients with acute lymphocytic leukemia (ALL), and shows that the miR-17-92 cluster, which contains both hsa-miR-18 and hsa-miR-20, is upregulated in ALL: "The miR-17-92 cluster seemed to be upregulated in all of our ALL samples. This cluster consists of … miR-18 … [and] miR-20…" *Id.,* at 1437-1438. In addition, as shown below, microarray expression analysis shows that Hep3b cell express both of these miRNAs.

miRNA name	**max_signal**	**average**	**std**	**number**
hsa-miR-18b	4930	4930	NULL	1
hsa-miR-18b	4268	1969.219512	740.4044	41
hsa-miR-20b	3113	2336.333333	793.2031581	9
hsa-miR-20b	3254	1581.875	574.9069345	16

Accordingly, both SEQ ID NO: 9 (hsa-miR-18) and SEQ ID NO: 2 (hsa-miR-20) are expressed in cells.

On page 12 of the Office Action, the Examiner suggests that miRNAs must be validated by analyzing miRNA expression patterns, as well as testing the effects of miRNA overexpression and underexpression. Applicant respectfully submits herewith experimental evidence that both claimed miRNAs, hsa-miR-18 (SEQ ID NO: 9) and hsa-miR-20 (SEQ ID NO: 2) regulate the levels of ATP7A and STAT3 mRNAs, respectively (further details are shown in Appendix A). As shown above, Hep3B cells express both of these miRNAs. Hep3B cells were transfected with anti-sense oligonucleotides (ASO) that specifically bind to hsa-miR-18 and hsa-miR-20, respectively, and the amount of their respective targets ATP7A and STAT3 was quantified using quantitative PCR, which is an established method in the art. *See* Fabani M.M. and Gait M.J. RNA 2008;14(2):336-46. The ASOs to hsa-miR-18 and hsa-miR-20 each led to increases in the levels of the mRNAs of their respective targets, as measured by cycle threshold.

Specifically, cells transfected with an ASO that targets hsa-miR-18 showed an approximately 1.8-fold increase in the level of ATP7A mRNA compared to no-ASO control cells. *See* Appendix A, Figure 2. Likewise, cells transfected with an ASO that targets hsa-miR-20 exhibited an approximately 1.2-fold increase in the level of STAT3 mRNA compared to no-ASO control cells. *See* Appendix A, Figure 1.

Accordingly, claimed sequences SEQ ID NO: 9 (hsa-miR-18) and SEQ ID NO: 2 (hsa-miR-20) are actually expressed in cells, and specifically inhibiting the activity of these miRNAs results in increased levels of their target mRNAs. Therefore, the utility of the claimed nucleic acids is not "merely speculation," but is firmly established by experimental evidence. In view of the foregoing, Applicant

respectfully requests that the Examiner reconsider and withdraw the rejection of the claims under 35 U.S.C. § 101. Additionally, because the claimed nucleic acids are supported by a specific, substantial, and credible utility, Applicant requests that the Examiner reconsider and withdraw the rejection of the claims under 35 U.S.C. § 112, first paragraph.

3. Conclusion

Applicant respectfully submits that the instant application is in good and proper order for allowance and early notification to this effect is solicited. If, in the opinion of the Examiner, a telephone conference would expedite prosecution of the instant application, the Examiner is encouraged to call the undersigned at the number listed below.

Respectfully submitted,

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APPENDIX A

Target validation for hsa-miR-20b and hsa-miR-18b

In order to validate **STAT3** (SEQ ID NO. 55282, RefSeq NM_139276) as a target of **hsa-miR-20b** (SEQ ID NO. 2)**,** Applicant transfected Hep3B cells which were shown to express **hsa-miR-20b** as was detected by microarray analysis (shown in Table 1), with specific anti-sense oligonucleotides (ASOs) to **hsa-miR-20b.**

Table 1

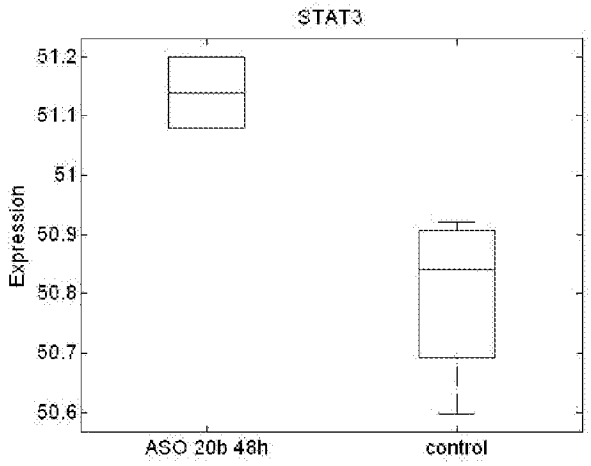
sourceName	miRNA name	max_signal	average	std	number
Hep3B2.1-7	hsa-miR-18b	4930	4930	NULL	1
Hep3B2.1-7	hsa-miR-18b	4268	1969.219512	740.4044	41
Hep3B2.1-7	hsa-miR-196b	1051	1051	NULL	1
Hep3B2.1-7	hsa-miR-20b	3113	2336.333333	793.2031581	9
Hep3B2.1-7	hsa-miR-20b	3254	1581.875	574.9069345	16

Hep3B cells were transfected with ASO to miR-20b, for 24 and 48 hours. After transfection RNA was isolated and mRNA of STAT3 was quantified using specific primers by SYBR RT-qPCR method (see MATERIALS AND METHODS section bellow).

Measuring the amount of initial mRNA was based on the observation that the amount of cDNA generated from the mRNA doubles with every cycle of PCR. Therefore, after N cycles, there is 2N times as much. In order to quantify the initial amount of mRNA, the cycle number at which the increase in fluorescence (and thus the amount of cDNA) was exponential, was measured. A threshold at this level of fluorescence was set. This threshold is indicated as the cycle threshold, or Ct. To compare the differences in quantity between a specific mRNA in two different samples, the Ct was calculated in each of the samples, and the delta Ct (dCT) was calculated. The fold-change between the amount of mRNA in the two samples was represented by 2^{dCt}.

STAT3 expression with and without ASO to miR-20b is presented in Figure 1:

Figure 1



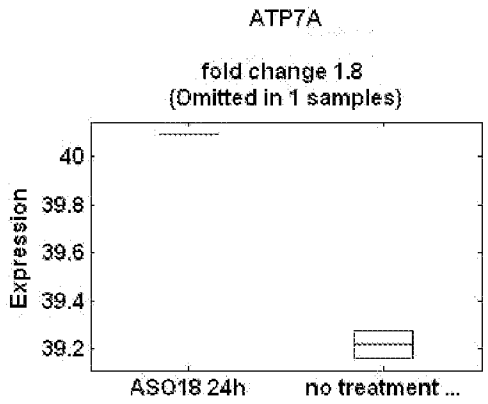
As shown in Figure 1, the fold change for the target STAT3 while knocking down miR-20b was approximately 1.2 (*i.e.*, $2^{dCt} = 2^{(51.15-50.85)}$). The term "control" refers to untransfected cells (which express miR-20b).

Figure 1 clearly shows that knocking down hsa-miR-20b by an ASO, caused a significant increase in the level of STAT3 mRNA, indicating that this miRNA regulates STAT3. These results support the utility disclosed in the application as filed.

In order to validate **ATP7A** (RefSeq NM_000052.1) as a target of **hsa-miR-18b** (SEQ ID NO. 9)**,** Applicant transfected Hep3B cells which were shown to express **hsa-miR-18b** as was detected by microarray analysis (attached), with specific anti sense oligonucleotids (ASOs) to **hsa-miR-18b.** Hep3B cells were transfected with ASO to miR-18b, for 24 and 48 hours. After transfection RNA was isolated and mRNA of **ATP7A** was quantified using specific primers by SYBR RT-qPCR method (see MATERIALS AND METHODS section bellow).

ATP7A expression with and without ASO to miR-18b is presented in Figure 2:

Figure 2



As shown in Figure 2, the fold change for the target ATP7A in the absence of miR-18b was approximately 1.8 (*i.e.*, $2^{dCt} = 2^{(40.05-39.20)}$). The term "control" refers to untransfected cells (which express miR-18b). Figure 2 clearly shows that knocking down hsa-miR-18b by an ASO, caused a significant increase in the level of ATP7A mRNA, indicating that this miRNA regulates this target gene.

MATERIALS AND METHODS

Transfection and treatment of cells

Hep3B cells from the American Type Culture Collection (ATCC, Rockville, MD) were plated in 6-well plates 24 hrs prior to transfection or treatment. Cells were transfected with specific anti sense oligonucleotids (ASO's) to microRNA, from IDT, using Oligofectamine (Invitrogen, Carlsbad, CA)).

RNA Isolation and Reverse Transcription

Total RNA was isolated by EZ-RNA II kit (Biological Industries) 24 and 48 hrs after transfection. 1µg of total RNA was reverse transcribed using Superscript II.

Quantification by RT-qPCR

mRNA was quantified by real-time-qPCR SYBR Green method, using 7500 Fast Real time PCR system, AB applied Biosystems. Each test was done in triplicates. Ct values were normalized to TBP as a house keeping gene.

			Primers for Target	
miRNA	**Target**	**ASO for miRNA**	**Fwd**	**Rev**
hsa-miR-20b	STAT3	CTACCTGCACTATGAGCACTTTG	GGCCATCTTGAGCACTAAGC	TCTGGCCGACAATACTTTCC
hsa-miR-18b	ATP7A	CTAACTGCACTAGATGCACCTTA	TGTGATGGCTGGCAATGATG	CTTCGACCGACAAACCTGAG
			CAGGTTTGTCGGTCGAAGTG	TTTCTGCCGATGTCTTCGAG
House keeping	TBP		TATAATCCCAAGCGGTTTGC	CACAGCTCCCCACCATATTC

STATISTICAL ANALYSIS

The statistical method used was a t-test (two-sided unpaired t-test) between the negative control and treated samples. In addition normalization was done by subtracting the Ct value of a house keeping gene-TBP. Ct values were determined using a default threshold of 0.2 in the 7500 Fast Real time PCR system, by ABI.